Genome analysis

Detection of DNA copy number alterations using penalized least squares regression

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ABSTRACT
Motivation: Genomic DNA copy number alterations are characteristic of many human diseases including cancer. Various techniques and platforms have been proposed to allow researchers to partition the whole genome into segments where copy numbers change between contiguous segments, and subsequently to quantify DNA copy number alterations. In this paper, we incorporate the spatial dependence of DNA copy number data into a regression model and formalize the detection of DNA copy number alterations as a penalized least squares regression problem. In addition, we use a stationary bootstrap approach to estimate the statistical significance and false discovery rate.

Results: The proposed method is studied by simulations and illustrated by an application to an extensively analyzed dataset in the literature. The results show that the proposed method can correctly detect the numbers and locations of the true breakpoints while appropriately controlling the false positives.

Availability: http://bioinformatics.med.yale.edu/DNACopyNumber

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Supplementary Information: http://bioinformatics.med.yale.edu/DNACopyNumber

1 INTRODUCTION

Genomic DNA copy number alterations are characteristic of many human diseases including cancer, where deletions and amplifications of DNA can contribute to alterations in the expression of tumor-suppressor genes and oncogenes, respectively. Therefore, the identification of DNA copy number alterations is important in understanding the genesis and progression of human cancers (Lengauer et al., 1998). Various techniques and platforms have been developed for genome-wide analysis of DNA copy number, such as comparative genomic hybridization (CGH) (Kallioniemi et al., 1992), array-based comparative genomic hybridization (aCGH) (Pinkel et al., 1998; Snijders et al., 2001), representational difference analysis (RDA) (Lisitsyn et al., 1993) and commercially available single nucleotide polymorphism (SNP) arrays (Zhao et al., 2004).

The goal of the analysis of DNA copy number data is to partition the whole genome into segments where copy numbers change between contiguous segments, and subsequently to quantify the copy number in each segment. Therefore, identifying the exact locations of copy number changes is fundamentally important to the analysis of DNA copy number data. Many statistical methods have been developed to address this issue. Jong et al. (2003) developed a genetical local search algorithm to best localize the breakpoints along the chromosome. Olshen et al. (2004) proposed a modified binary segmentation procedure, called circular binary segmentation (CBS), to look for two breakpoints at a time by considering the segment as a circle. Fridlyand et al. (2004) used an unsupervised hidden Markov model (HMM) approach to classify each chromosome into different states representing different copy numbers. Wang et al. (2005) proposed a hierarchical clustering algorithm, called ‘cluster along chromosomes’ (CLAC), to select interesting clusters by controlling the false discovery rate (FDR, Benjamini and Hochberg, 1995; Storey, 2002). Hsu et al. (2005) used a wavelets approach to denoising the data to uncover the true copy number changes. Lai and Zhao (2005) used the t-test to detect copy number alterations by aggregating information from replicated arrays. More recently Price et al. (2005) applied dynamic programming to search for breakpoints, and Picard et al. (2005) further combined dynamic programming with penalized likelihood to identify breakpoints.

In this paper, we propose a novel approach to assess DNA copy number alterations based on the penalized least squares method. Let us consider an array CGH profile, and denote $Y_i$ as the log2 ratio of the intensities of the red over green channels of marker $i$ on a chromosome where the red and green channels measure the intensities of the cancer and normal samples. We further assume that the observed $Y_i$ is a realization of the true relative copy number $\beta_i$ at marker $i$ plus a random noise,

$$Y_i = \beta_i + \epsilon_i, \quad i = 1, \ldots, n,$$

where $n$ is the number of markers on a given chromosome. Note that the copy number data are ordered by the locations of the markers and have spatial dependence due to the physical dependence of nearby markers. In fact, the spatial dependence of the copy number data is exhibited in both signals $\beta_i$ and noises $\epsilon_i$. The signals $\beta_i$ have spatial dependence because the true copy numbers of the nearby markers are the same except in the regions where the copy numbers change abruptly. In Figure 1, we illustrate the spatial dependence of...
noises on chromosome 1 of two cell lines from the Coriel dataset (Snijders et al., 2001). Similar to the analysis of time-series data, we use the partial auto-correlation function (PACF, Brockwell and Davis, 1996) to characterize the spatial dependence, though the markers are not equally spaced along the chromosome. Figure 1(a) and (b) depict a normal chromosome 1 (GM05296) and its corresponding PACF. Figure 1(c) depicts an abnormal chromosome 1 (GM13330). After centering the altered segment (the dots on the right side of the vertical line) to have mean zero, Figure 1(d) depicts the PACF of the centered data. Figure 1(b) and (d) demonstrate the spatial dependence of noise in copy number data. It is desirable and necessary to utilize both types of spatial dependences of the signal and noise in statistical inference.

The remainder of the article is organized as follows. In the Methods section, we incorporate the spatial dependence of signals into model (1) and formalize it as a penalized least squares regression problem. In addition, we consider the spatial dependence of noises and use a stationary bootstrap approach to estimating P-value and FDR. In the Results section, the proposed methods are evaluated by a simulation study and illustrated by an application to an extensively studied dataset. We summarize the results and discuss future research directions in the Discussion section.

2 METHODS

2.1 Penalized least squares regression

The signals $\beta_i$ have spatial dependence due to the physical dependence of nearby markers. Intuitively, $\sum_{j=1}^{n-1} |\beta_{i+j} - \beta_i|$ provides a measure of the smoothness of the parameters $\beta_i$, which essentially reflects the spatial dependence of the signals. Hence, we propose to estimate $\beta_i$ by

$$\arg\min \sum_{i=1}^{n} (Y_i - \beta_i)^2 \quad \text{subject to} \sum_{i=1}^{n-1} |\beta_{i+1} - \beta_i| < s,$$

where $s$ is a tuning parameter. The global smoothness of the parameters is controlled by the constraint $\sum_{i=1}^{n-1} |\beta_{i+1} - \beta_i| < s$. Set $\beta_0 = 0$, and define $\mu_i = \beta_i - \beta_{i-1}$, which can be interpreted

![Fig. 1. Spatial dependence of noises in DNA copy number data. GM05296 and GM13330 are two cell lines from the Coreil dataset. (a) A normal chromosome 1 without alterations. (b) The corresponding partial auto correlation function of chromosome 1 on GM05296. (c) A chromosome 1 with alterations. Centering the dots (those on the right side of the vertical line) around zero yields the triangles. (d) The partial auto correlation function of the centered data.](https://academic.oup.com/bioinformatics/article-abstract/21/20/3811/203554/1203556)
as the jump between the \((j-1)\)th and \(j\)th markers. Then model (1) can be transformed into the following model

\[
Y_i = \sum_{j=1}^{m} \mu_j + \epsilon_i, \quad i = 1, \ldots, n. \tag{2}
\]

The parameters \(\mu_j\) can be estimated by

\[
\arg \min_{\mu} \sum_{i=1}^{n} (Y_i - \sum_{j=1}^{m} \mu_j)^2, \quad \text{subject to } \sum_{j=2}^{n} |\mu_j| < s. \tag{3}
\]

Problem (3) is a penalized least squares regression with \(L_1\) penalty, and also referred to as Lasso regression (Tibshirani, 1996; Efron et al., 2004) in model selection. Henceforth, we call the proposed method the Lasso-based (LB) method. For a fixed \(s\), the solution for problem (3) can be obtained by using standard quadratic programming. Efron et al. (2004) demonstrated that Lasso belongs to a more generalized model selection algorithm, called ‘Least Angle Regression’ (LARS), which is a less aggressive version of forward stepwise regression. LARS exploits the geometry of the algorithm, and requires the same order of computational effort as ordinary least squares. A simple modification of the LARS algorithm calculates the entire path of Lasso solutions (for all values of \(s\)), which are piecewise linear functions of \(s\).

The Lasso regression results in a soft thresholding rule which shrinks some coefficients to zero (Donoho and Johnstone, 1994). The tuning parameter \(s\) controls the sparsity of the solution. The smaller the \(s\), the more sparse the solution and more parameters are shrunk to zero. Sparsity is desirable from both statistical and biological points of view, which can reduce the complexity of the model and also requires that the copy number of the altered region has to be substantially larger than a threshold. (We note that it is possible to utilize the SCAD penalty proposed by Fan and Li, 2001, Antoniadis and Fan, 2001, which also has the sparsity property, though we do not pursue them here further.)

Choosing \(s\) is like choosing the number of breakpoints. Denote \(A\) as the active set of breakpoints. As \(s\) is varied from 0 to infinity, Lasso algorithm adds or removes one breakpoint at a time from \(A\). Correctly choosing \(s\) is crucial because if \(s\) is too large, the LB method may detect more false positives; and if the selected \(s\) is too small, the LB method may not detect all the true breakpoints. In this paper, we empirically choose \(s\) in the following way:

1. Starting with all coefficients equal to zero, we have \(s = 0\) and \(A = \emptyset\).
2. When one additional covariate (i.e. a breakpoint) is added into the model, the corresponding breakpoint will partition a particular segment into two subsegments. If both of the following conditions (a) and (b) are satisfied, \(s\) is updated as the \(L_1\) norm of coefficients of the current model. Otherwise, we stop.
   (a) The difference of the means of the two subsegments is \(>0.35\).
   (b) At least one of the subsegments has a mean \(>0.35\).

The threshold .35 is chosen because the absolute log2 ratio is .35 when the copy number is increased or decreased by about half, \(\log_2(2.55/2) = -\log_2(1.57/2) = 0.35\). In practice, we repeat step 2 up to ten times, because the number of true breakpoints is small and Lasso adds them into \(A\) in the first few steps.

In model (2), the parameter \(\mu_j\) can be interpreted as the jump between the \((j-1)\)th and \(j\)th markers. A significant \(\mu_j\) corresponds to a true breakpoint at which the copy number is changed. In the next section, we propose a stationary bootstrap approach to test the significance of the parameters.

### 2.2 Estimation of \(P\)-value and FDR

There is no parametric testing method for Lasso regression due to the complexity of the algorithm. In addition, we do not specify a parametric distribution of the random noises in model (2). In the absence of parametric tests, permutation and bootstrap are two possible methods to infer the significance of the parameters. However, the presence of the spatial dependence in the DNA copy number data invalidates the exchangeability of the data and the simple global permutation is inappropriate in this context. In this paper, we propose to use the stationary bootstrap method for statistical inferences. We first assume that the \(Y_i\) follow the same distribution under the null hypothesis of no copy number alterations. If we further assume that the \(Y_i\) follow a weakly dependent stationary process, then we can resample the true null distribution using the stationary bootstrap method proposed by Politis and Romano (1994). The stationary bootstrap method resamples ‘blocks of blocks’ of observations of random length, where the length of each block follows a geometric distribution. More specifically, we first pick one observation randomly from the original observations, say \(Y_{i_1}\). Then with probability \(\theta\) we pick a new observation randomly from the original observations, say \(Y_{i_2}\). Note that \(i_1\) could be equal to \(i_2\). Or with probability \(1-\theta\) we pick the ‘next’ observation following current observation, and that is equivalent to picking \(Y_{i_1+i_2}\). Note that the ‘next’ observation following \(Y_{n}\) is \(Y_{1}\). This procedure is repeated \(n\) times to resample a new set of observations from the original observations. With the above stationary bootstrap method, we propose the following procedure to calculate the \(P\)-value and FDR.

Step 1: Given the original observations \(\{Y_i\}_{i=1}^n\), denote \(\hat{\mu}_i\) as the Lasso solution to (3). All non-zero \(\hat{\mu}_i\) indicate putative breakpoints which partition a chromosome into segments. For each segment, center \(Y_i\) around zero by subtracting the average copy number of the segment, and denote the centered data as \(\{Y_i'\}_{i=1}^n\).

Step 2: Given the centered data \(\{Y_i'\}_{i=1}^n\), resample \(N\) sets of observations using the above stationary bootstrap method.

Step 3: For a set of bootstrapped observations, say the \(k\)th, denote \(\hat{\mu}_{ik}\) as the Lasso solution to (3).

Note that the same \(s\) is used in Steps 1 and 3 in order to derive the correct distribution. Given a large number of resamplings, say \(N = 1000\), the distribution of \(\hat{\mu}_k\) under the null hypothesis can be approximated by the marginal distribution of \(\{\hat{\mu}_{ik}\}_{k=1}^N\). The \(P\)-value for the observed \(\hat{\mu}_i\) can be estimated by

\[
P_i = \frac{\#\{\hat{\mu}_{ik} \geq \hat{\mu}_i, k = 1, \ldots, N\}}{N} + \frac{\#\{|\hat{\mu}_{ik}| = |\hat{\mu}_i|, k = 1, \ldots, N\}}{2N},
\]

\(i = 1, \ldots, n\).
where \# represents the number of elements in a set. As for the estimate of FDR, for a given cutoff value, say $p = 0.005$, it can be estimated by

$$\text{FDR} = \frac{p \times \text{total number of markers}}{\text{number of markers whose P-values are less than } p}.$$

As pointed out by Fan et al. (2004), this FDR estimation method is equivalent to the Benjamini and Hochberg (1995) method with the empirical control of the FDR.

A practical issue of the stationary bootstrap approach is the selection of $\theta$. Note that the stationary bootstrap method becomes the classic bootstrap method (sample with replacement) when the \(Y_i\) are independent. Politis and Romano (1994) pointed out that the selection of $\theta$ is essentially a ‘smoothing’ problem, and it is difficult to choose $\theta$ optimally. For real data analysis and simulations in the next selection, we tried a number of values ($\theta = 0.05, 0.1, 0.25, 0.35, 0.5$) to test the robustness of the LB method to the selection of $\theta$. The results showed that the LB method is robust to the choice of $\theta$ (results not shown). The results presented in the next section were obtained using $\theta = 0.25$.

### 3 RESULTS

#### 3.1 Application to BAC array

To evaluate the LB method, we first apply it to a BAC array dataset with experimentally tested DNA copy number alterations (Snijders et al., 2001). The dataset was also used by Olshen et al. (2004), Fridlyand et al. (2004), Wang et al. (2005), Hsu et al. (2005) and others to evaluate their methods. The dataset consists of single experiments on 15 fibroblast cell lines. Each array contains measurements for 2700 BACs spotted in triplicates. There were 15 chromosomes with partial alterations and 8 whole chromosomal alterations. All but one of these alterations were confirmed by spectral karyotyping [Chromosome 15 on GM07801, see Figure 3(b)].

The LB method identified all 14 partial chromosomal alterations confirmed by Snijders et al. (2001), and no chromosomal alteration for chromosome 15 on GM07801, given the cutoff $P$-value 0.005. In comparison with the LB method, HMM of Fridlyand et al. (2004) and CBS of Olshen et al. (2004) could not detect narrow regions at

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the telomeric ends (Chromosome 9 on GM03563 and Chromosome 12 on GM01535). The HMM method defined these regions as focal aberrations and placed them into an abnormal state. Figure 2 illustrates four chromosomes identified by the LB method among the 14 chromosomes. In addition, the LB method found seven other chromosomes with alterations which have not been confirmed by spectral karyotyping. Five of them are single marker alterations. The other two are sex chromosomes 23 (GM01535 and GM07081) which showed whole chromosome alterations except the single marker in the telomeric regions. These additional identifications are summarized in Figure 3 (a)–(g). They could be false positives, or represent real DNA copy number alterations which are undetectable due to the low resolution of spectral karyotyping. In fact, HMM of Fridlyand et al. (2004) also detected a number of unconfirmed single-marker aberrations. When we ran HMM on these seven chromosomes, it detected the same breakpoints as the LB method. CBS of Olshen et al. (2004) could not detect single-marker aberrations, but still found a number of chromosomes with alterations which were not confirmed by spectral karyotyping. The authors argued that these additional identifications were a result of local trends in the data.

Table 1. Comparison of the performance of three methods for data generated under model (5). For each method, the first row lists the average number of detected breakpoints along with the estimated standard deviations; the second row lists the average number of detected breakpoints within and beyond 2 markers of the true breakpoints, respectively.

<table>
<thead>
<tr>
<th>Method</th>
<th>1000 markers</th>
<th>500 markers</th>
<th>250 markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>5.30 (0.66)</td>
<td>5.50 (0.89)</td>
<td>5.90 (1.02)</td>
</tr>
<tr>
<td></td>
<td>5.10, 0.20</td>
<td>5.35, 0.15</td>
<td>5.65, 0.25</td>
</tr>
<tr>
<td>HMM</td>
<td>10.85 (7.28)</td>
<td>7.60 (2.68)</td>
<td>5.75 (1.07)</td>
</tr>
<tr>
<td></td>
<td>4.95, 5.90</td>
<td>4.95, 2.65</td>
<td>5.10, 0.65</td>
</tr>
<tr>
<td>CBS</td>
<td>40.60 (6.71)</td>
<td>13.95 (4.62)</td>
<td>5.85 (1.14)</td>
</tr>
<tr>
<td></td>
<td>5.00, 35.60</td>
<td>5.00, 8.95</td>
<td>5.00, 0.85</td>
</tr>
</tbody>
</table>

3.2 Simulation
In this section, we investigate the performance of the LB method through simulations. Suppose there are 1000 markers equally spaced along a chromosome, under the null hypothesis of no
DNA copy number alterations, the log2 ratios of these 1000 markers are simulated from an AR(2) model as follows:

$$e_i = \alpha_1 e_{i-1} + \alpha_2 e_{i-2} + \epsilon_i, \quad i = 1, \ldots, 1000,$$

where \((\alpha_1, \alpha_2) = (0.6, 0.2)\) and \(\epsilon_i \sim N(0, 0.1^2)\). We then assume that there are three altered regions along the chromosome which correspond to quadruploid, triploid and monoploid states, respectively. More specifically, the true log2 ratios of 1000 markers are generated as follows:

$$Y_i = \mu_i + \epsilon_i, \quad i = 1, \ldots, 1000,$$

where the \(\mu_i\) are defined in the following table,

<table>
<thead>
<tr>
<th>(i)</th>
<th>1–100</th>
<th>101–150</th>
<th>151–450</th>
<th>451–600</th>
<th>601–900</th>
<th>901–1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_i)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.585</td>
<td>0</td>
<td>-1</td>
</tr>
</tbody>
</table>

To consider the density of markers along the chromosome, we create two subsets containing 500 and 250 markers, respectively, by drawing one marker from every two and four markers. We then simulate 20 datasets from the above model, and apply the LB, HMM and CBS methods to estimate the numbers and locations of the breakpoints. For the LB method, we choose the cutoff \(P\)-values as 0.001, 0.002 and 0.004, respectively, to control the number of false positives to be at most 1. For the CBS method, we choose the same cutoff \(P\)-values, and also use the ‘prune’ procedure to reduce the number of false positives. For the HMM method, we use the Akaike information criterion (AIC) and set the minimum difference of merging two states to be 0.35. Table 1 presents the simulation results.

For each method, the first row lists the average number of detected breakpoints (and the corresponding estimated standard deviation). It shows that the LB method performs quite robustly for different number of markers. CBS tends to detect more false positives when the density of markers becomes higher. In fact, Olshen et al. (2004) showed that CBS might detect false positives even when the density of markers and the noise level are low. Though HMM can accurately estimate the number of hidden states, it might not correctly partition the chromosome. It tends to detect more false positives when the signal-to-noise ratio is low. To further evaluate the accuracy of localizing the breakpoints, we also calculate the average number of detected breakpoints within and beyond two markers of the true breakpoints, and list the results in the second row. It shows that the breakpoints detected by the LB method are very close to the true breakpoints. (More details are provided in the Supplementary website.)

Furthermore, we run two other similar simulations to study the dependence assumption on the three methods. More specifically, instead of simulating \(\epsilon_i\) from an AR(2) model, we generate them either independently

$$e_i = \epsilon_i, \quad i = 1, \ldots, 1000,$$

or from an AR(1) model:

$$e_i = 0.6d_{i-1} + \epsilon_i, \quad i = 1, \ldots, 1000,$$

where \(\epsilon_i \sim N(0, 0.154^2)\) and \(\epsilon_i \sim N(0, 0.123^2)\). Note the standard deviation of \(e_i\) is the same for these three models. Table 2 summarizes the results, which show that the LB method is quite robust to the dependence assumption. In contrast, the HMM and CBS methods are sensitive to the dependence assumption, and tend to detect more false positives when the dependence between the nearby markers becomes stronger.

### 4 DISCUSSION

In this paper, we have proposed a new approach, the LB method, to assess DNA copy number alterations along the chromosome. The LB method was applied to an aCGH dataset, and was able to detect all the alterations confirmed by spectral karyotyping. Through simulations we demonstrated that the LB method can correctly infer the numbers and locations of the true breakpoints while appropriately controlling the false positives.

The LB method is conceptually simple and easy to interpret, and may be useful in other genomic problems where the data have spatial dependence structure, such as tiling arrays (Bertone et al., 2004). It showed better performance in comparison with the HMM and CBS methods. The LB method is quite robust when the marker density becomes higher and the spatial dependence of noises becomes stronger, where the HMM and CBS methods tend to detect more false positives. In fact, CBS does not utilize the spatial information and assumes that markers are independent, and HMM has no inference feature and simply partitions the genome based on the hidden state of the markers.

The LB method estimates the parameters through Lasso regression, and performs well for a moderate dataset. When the number of markers is large, we divide the data into a number of overlapping windows of equal size and progress the LB method within each to facilitate the computation of Lasso regression. All the numerical analyses are done using R, and the program can be downloaded at [http://bioinformatics.med.yale.edu/DNACopyNumber](http://bioinformatics.med.yale.edu/DNACopyNumber). It took 9 CPU minutes to analyze a chromosome with 1000 markers on a Dell Pentium4 PC.

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**Table 2.** Comparison of the performance of three methods for models (6) and (7).

<table>
<thead>
<tr>
<th>Method</th>
<th>Independent model number of markers</th>
<th>AR(1) model number of markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>LB</td>
<td>5.95 (0.89)</td>
<td>6.15 (0.88)</td>
</tr>
<tr>
<td></td>
<td>5.70 (0.25)</td>
<td>6.05 (0.10)</td>
</tr>
<tr>
<td>HMM</td>
<td>6.10 (1.68)</td>
<td>6.05 (1.43)</td>
</tr>
<tr>
<td></td>
<td>5.15 (0.95)</td>
<td>5.25 (0.80)</td>
</tr>
<tr>
<td>CBS</td>
<td>5.00 (0.00)</td>
<td>5.05 (0.22)</td>
</tr>
<tr>
<td></td>
<td>4.95 (0.05)</td>
<td>5.00 (0.05)</td>
</tr>
<tr>
<td></td>
<td>5.15, 0.95</td>
<td>5.25, 0.65</td>
</tr>
<tr>
<td></td>
<td>5.00, 1.10</td>
<td>5.00, 1.10</td>
</tr>
<tr>
<td></td>
<td>4.90, 11.70</td>
<td>5.00, 0.85</td>
</tr>
<tr>
<td></td>
<td>4.95, 0.25</td>
<td>5.00, 0.20</td>
</tr>
</tbody>
</table>
In the future, we would like to incorporate the distance between markers into model (2), as the current model implicitly assumes that the markers are equally spaced along the chromosome. We would also like to refine the selection of tuning parameter $s$ as well as the dependence parameter $\theta$ in the stationary bootstrap in order to reduce the false positives and apply the LB method to even larger datasets, such as 50K and 100K SNP array data. Another important issue to be considered is extending the proposed model to efficiently utilize the replicate information as studied in Lai and Zhao (2005).

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Conflict of Interest: none declared.

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